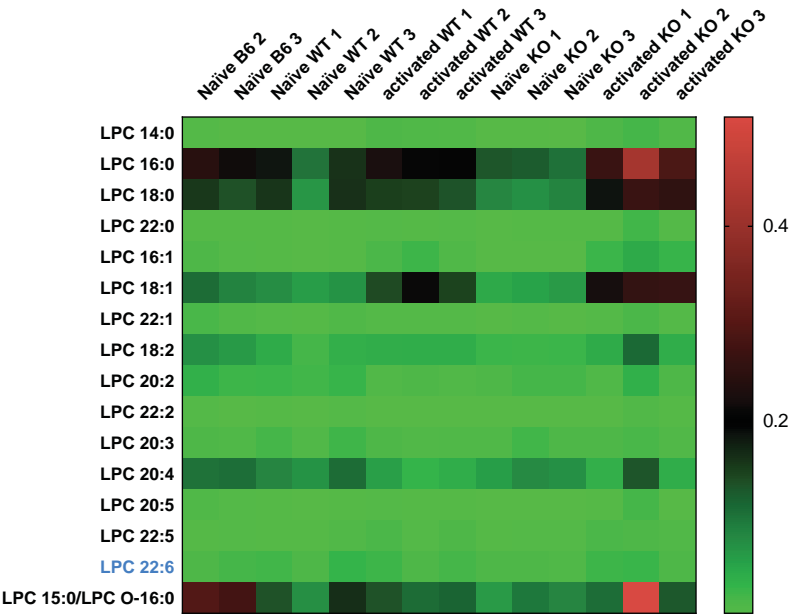
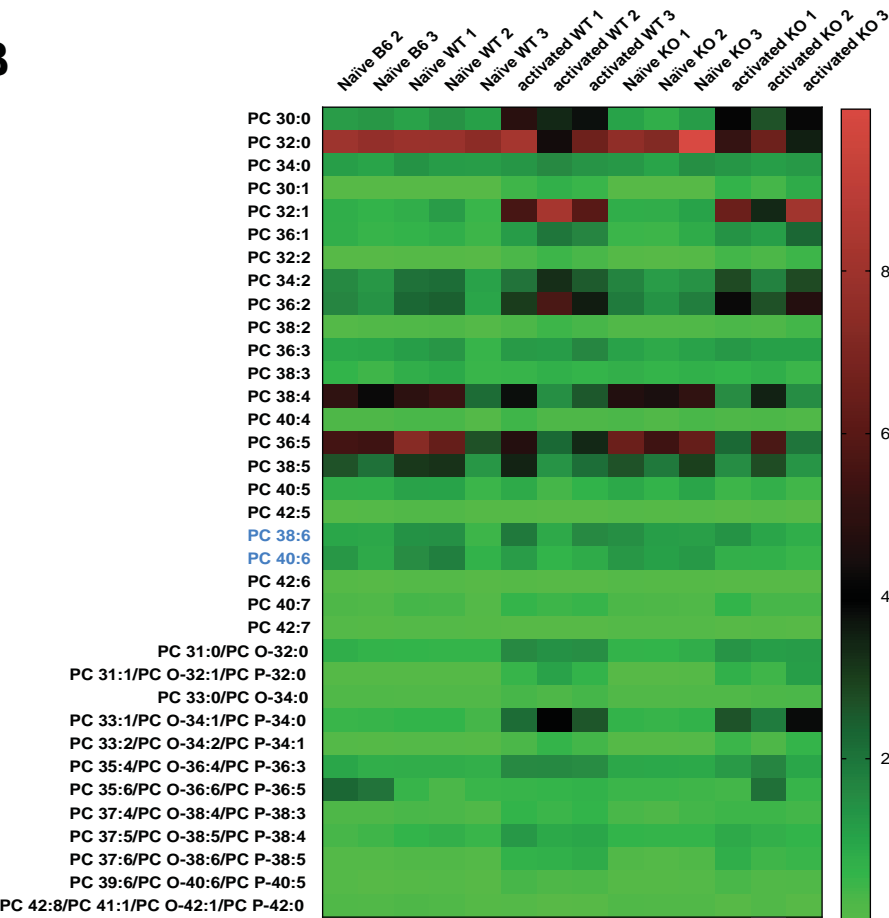


Supplemental Figure 1

A

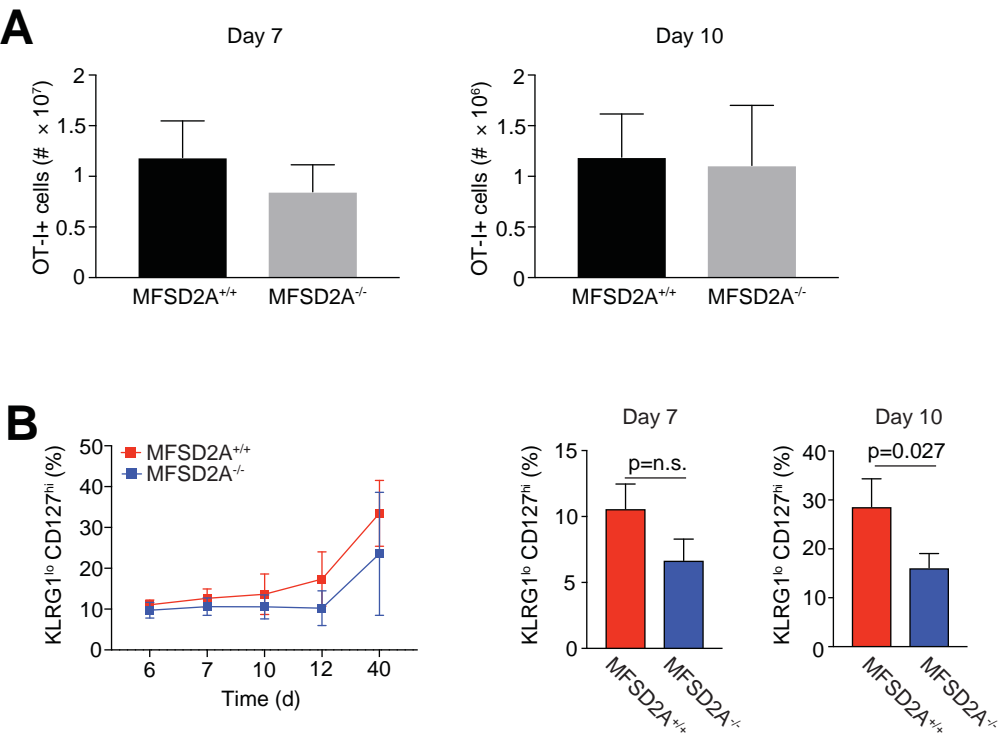


B



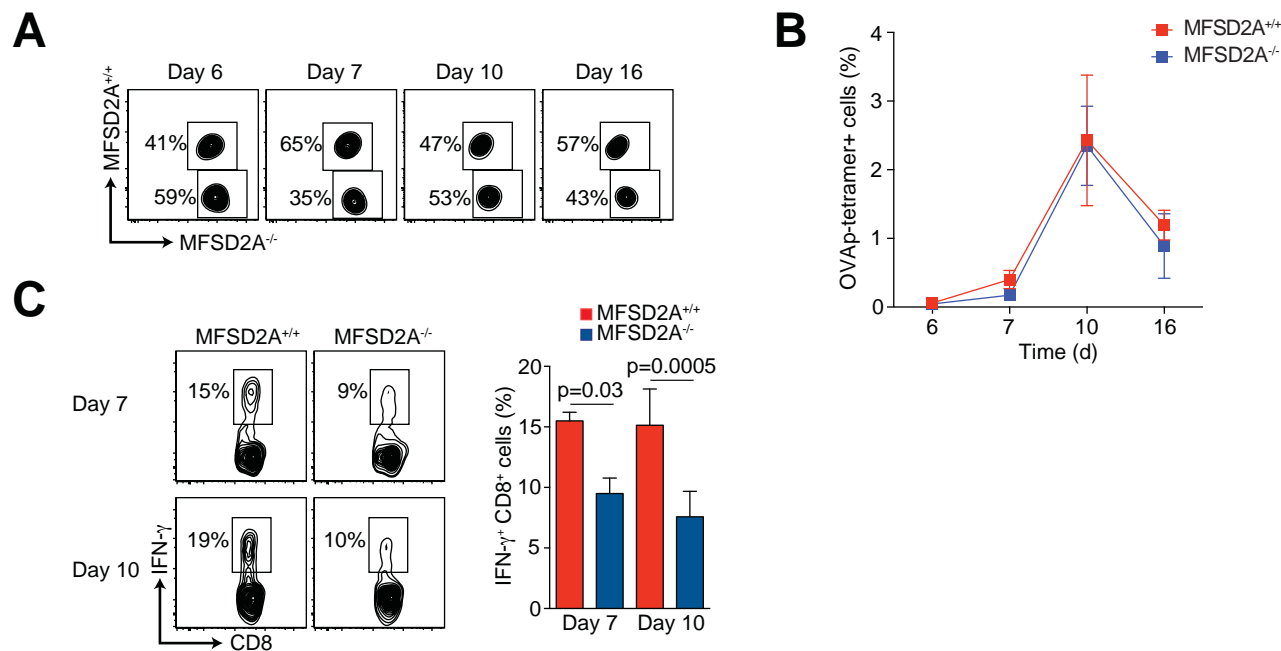
Supplemental Figure 1. Lipidomic analysis of naïve and in vitro activated MFSD2A-deficient CD8⁺ T cells. Heatmap representation of percentage lysophosphatidylcholine (LPC) (A) and phosphatidylcholine (PC) (B) fatty acid species identified in naïve or activated MFSD2A^{+/+} and MFSD2A^{-/-} CD8⁺ T cells. Phospholipid species were calculated as a percentage of the total level of phospholipids. DHA-containing species are highlighted in blue. Each sample represents a biological replicate as indicated.

Supplemental Figure 2



Supplemental Figure 2. Phenotype of MFSD2A-deficient CD8+ T cells during primary infection. (A) Graphs indicate the number of OT-I CD8+ T cells isolated from the spleen of *Listeria*-OVA infected mice on days 7 and 10 after infection. (B) Graph (right) indicates the frequency of KLRG1^{lo} CD127^{hi} expression of transferred OT-I cells in the PBL over time after infection with *Listeria*-OVA. Bar graphs (center and left) indicate the frequency of KLRG1^{lo} CD127^{hi} expression of transferred OT-I cells isolated from the spleen on day 7 and 10 after infection with *Listeria*-OVA. Error bars show average and SEM. Data are representative of three experiments with three mice per group.

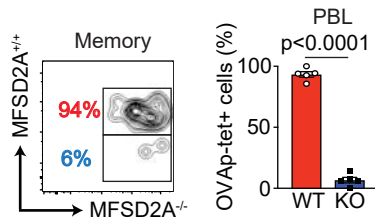
Supplemental Figure 3



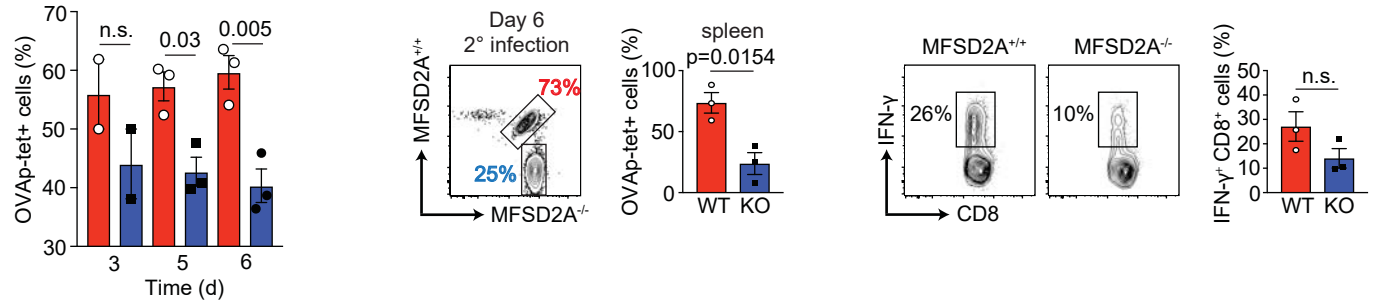
Supplemental Figure 3. Endogenous MFSD2A deficiency results in decreased effector function in the endogenous immune response. (A and B) Flow cytometry plots and graph showing the frequency of MFSD2A^{+/+} (CD45.1.2) and MFSD2A^{-/-} (CD45.2) OVA-tetramer⁺ T cells from the PBL of 1:1 mixed bone marrow chimeric mice at the indicated time point after infection. (C) Flow cytometry plots and graph showing IFN- γ intracellular staining on gated CD8⁺ CD44⁺ T cells from day 7 and day 10 infected mixed bone marrow chimeras. Data are representative of four independent experiments with 2-3 mice per timepoint per experiment. Error bars show average and SEM. P values were calculated using the student's t-test.

Supplemental Figure 4

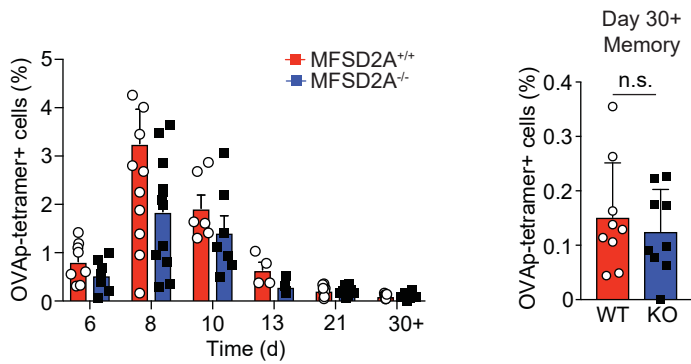
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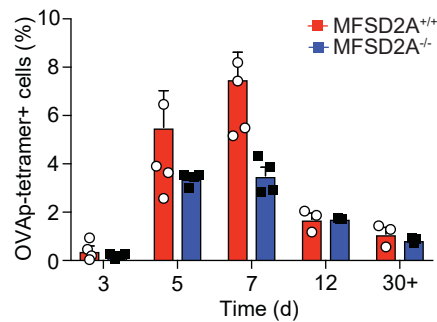
B



C



D



Supplemental Figure 4. MFSD2A deficiency results in decreased endogenous memory response under competitive conditions. (A) Flow plot and graphs indicating the frequency of MFSD2A^{+/+} (CD45.1.2) and MFSD2A^{-/-} (CD45.2) gated CD8⁺ OVA-tetramer⁺ T cells from the PBL of 1:1 mixed bone marrow chimeric mice at memory (day 40) after infection. (B) Graphs showing the frequency of MFSD2A^{+/+} (CD45.1.2) and MFSD2A^{-/-} (CD45.2) gated CD8⁺ OVA-tetramer⁺ T cells from the PBL of 1:1 mixed bone marrow chimeric mice during secondary response at the indicated timepoints (left). Flow cytometry plot and graph indicating the frequency of MFSD2A^{+/+} (CD45.1.2) and MFSD2A^{-/-} (CD45.2) T cells in the spleen on day 6 of secondary infection (center). Flow plots and graph indicating the frequency of IFN-γ-expressing cells in the spleen at day 6 after secondary infection. (C) Graphs showing the frequency of OVA-tetramer⁺ T cells from the PBL of MFSD2A^{+/+} or MFSD2A^{-/-} infected mice at the indicated time point after infection. Each dot represents an individual animal. (D) Graph showing the frequency of OVA-tetramer⁺ T cells from the PBL of MFSD2A^{+/+} or MFSD2A^{-/-} infected mice at the indicated time point after secondary infection with high dose Listeria-OVA. Each dot represents an individual animal. Data are representative of two independent experiments with 1-2 mixed bone marrow chimeras per experiment (A-B) or three independent experiments with at least 3 mice per group (C-D). Each dot represents one individual animal. Error bars show average and SEM. P values were calculated using the student's t-test or using one way ANOVA adjusted for multiple comparisons.